

STIC-ILL

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From: Canella, Karen
Sent: Tuesday, May 13, 2003 9:57 PM
To: STIC-ILL
Subject: ill order 09/520,489

Art Unit 1642 Location 8E12(mail)

Telephone Number 308-8362

Application Number 09/520,489

1. Biochemical and Biophysical Research Commun:
1989, 158(1):155-162
1991 Jan 31, 174(2):758-766
2. Lymphokine Research, 1989, 8(2):99-106
3. Immunology, 1996 Jan. 87(1):127-133
4. Cancer Immunol Immunother, 1986, 23(1):60-66
5. FEBS Letters, 1995, 372(1):44-48

L2 ANSWER 1 OF 1 MEDLINE

ACCESSION NUMBER: 94259184 MEDLINE

DOCUMENT NUMBER: 94259184 PubMed ID: 7911089

TITLE: pp60v-src kinase overexpression leads to cellular resistance to the antiproliferative effects of tumor necrosis factor.

AUTHOR: Aggarwal B B; Totpal K; Ali-Osman F; Budde R J; Pocsik E
CORPORATE SOURCE: Department of Clinical Immunology and Biological Therapy, University of Texas, M.D. Anderson Cancer Center, Houston 77030.

SOURCE: FEBS LETTERS, (1994 May 30) 345 (2-3) 219-24.
Journal code: 0155157. ISSN: 0014-5793.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199407

ENTRY DATE: Entered STN: 19940714

Last Updated on STN: 20000303

Entered Medline: 19940706

AB While some tumor cells are sensitive to the antiproliferative effects of tumor necrosis factor (TNF), others are resistant. The molecular basis for cellular resistance to TNF is not completely understood. Previously we have shown that transfection of cells with an oncogene HER2/neu/erb

B2, a receptor tyrosine kinase, leads to resistance to the anticellular effects of TNF [(1988) Proc. Natl. Acad. Sci. USA 85, 5102-5106]. In the present study, we demonstrate that the overexpression of another oncogenic tyrosine kinase, pp60v-src also induces resistance to TNF. In contrast to HER2, however, pp60v-src transfection of cells did not lead to

down-modulation of TNF receptors but rather to decreased intracellular glutathione levels. The pp60v-src-induced cellular resistance to TNF could be abrogated by interferon-gamma. Thus, these results indicate

that the resistance of certain tumors to TNF may also be due in part to the overexpression of pp60v-src oncogene.

L74 ANSWER 13 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1991:194298 BIOSIS
DOCUMENT NUMBER: BR40:91578
TITLE: PHASE I-II-TRIAL WITH **TNF-GAMMA**
INTERFERON IN **METASTATIC** RENAL CELL CARCINOMA.
AUTHOR(S): SOHN M; LEVENS W; RUEBBEN H; RICHTER R; RAETH U; KEMPENI
J;
CORPORATE SOURCE: JAKSE G
DEP. UROLOGY, UNIV. CLINICS RWTH AACHEN, PAUWELSSTRASSE,
5100 AACHEN, W. GER.
SOURCE: 15TH INTERNATIONAL CANCER CONGRESS, HAMBURG, GERMANY,
AUGUST 16-22, 1990. J CANCER RES CLIN ONCOL, (1990) 116
(SUPPL PART 2), 1058.
CODEN: JCROD7. ISSN: 0171-5216.
DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: English

L46 ANSWER 1 OF 1 PCTFULL COPYRIGHT 2003 Univentio
 ACCESSION NUMBER: 1996014328 PCTFULL ED 20020514
 TITLE (ENGLISH): **TUMOR NECROSIS FACTOR-
 GAMMA**
 TITLE (FRENCH): POLYPEPTIDE GAMMA APPARTENANT A LA FAMILLE DES
 FACTEURS
 INVENTOR(S): DE NECROSE TUMORALE (FNT)
 YU, Guo-Liang;
 NI, Jian;
 ROSEN, Craig, A.
 PATENT ASSIGNEE(S): HUMAN GENOME SCIENCES, INC.;
 YU, Guo-Liang;
 NI, Jian;
 ROSEN, Craig, A.
 LANGUAGE OF PUBL.: English
 DOCUMENT TYPE: Patent
 PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9614328	A1	19960517

DESIGNATED STATES
 W: AU CA CN JP KR NZ US AT BE CH DE DK ES FR GB GR IE IT
 LU MC NL PT SE
 APPLICATION INFO.: **WO 1994-US12880 A 19941107**
 TIEN **TUMOR NECROSIS FACTOR-GAMMA**
 AI **WO 1994-US12880 A 19941107**
 ABEN A human **TNF-gamma** polypeptide and DNA (RNA) encoding
 such polypeptide and a procedure for
 producing such polypeptide by recombinant techniques is disclosed.
 Also.
 . . certain cell types to treat diseases, for example restenosis.
 Also
 disclosed are diagnostic methods for detecting a mutation in the
TNF-gamma nucleic acid sequence or
 an overexpression of the **TNF-gamma** polypeptide.
 Antagonists against such polypeptides and their use
 as a therapeutic to treat cachexia, septic shock, cerebral malaria,
 inflammation, arthritis. . .
 DETD The TNF-gamma polypeptide may also be employed to
 treat autoimmune diseases such as Type I diabetes by
 enhancing the **T-cell proliferative**
 response.

L33 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1997:133820 CAPLUS

DOCUMENT NUMBER: 126:224097

TITLE: Production of prostaglandin E2 and collagenase is inhibited by the recombinant soluble **tumor necrosis factor** receptor p55-human . **gamma.3** fusion protein at concentrations a hundred-fold lower than those decreasing T cell activation

AUTHOR(S): Nicod, L. P.; Isler, P.; Chicheportiche, R.; Songeon, F.; Dayer, J.-M.

CORPORATE SOURCE: Rekspiratory Div., Univ. Hospital, Geneva, Switz.

SOURCE: European Cytokine Network (1996), 7(4), 755-763

CODEN: ECYNEJ; ISSN: 1148-5493

PUBLISHER: Libbey Eurotext

DOCUMENT TYPE: Journal

LANGUAGE: English

AB TNF-.alpha. and lymphotoxin .alpha. (TNF-.beta.) are pleiotropic cytokines

with regulatory functions in inflammatory reactions and T cell activation.

Natural TNF inhibitors such as sol. TNF-binding proteins, i.e. TNFsR55 and

TNFsR75, are shed from white blood cells and probably other cells. These naturally occurring inhibitors of TNF are shown to be 10 times less effective than the bivalent antagonist of TNF, recombinant sol. TNF receptors p55-human .gamma.3 fusion protein (rsTNFR-p55h.gamma.3), in controlling the release of prostaglandin E2 (PGE2) and collagenase by fibroblasts, as well controlling **T cell**

proliferation. To block the action of rhTNF-.alpha. added to fibroblasts, a fivefold excess of rsTNFR-p55h.gamma.3 was sufficient, but concns. of a hundred to a thousand times higher were required to obtain a significant inhibition of T cell activation. This concn. appears to be required to block membrane-bound TNF-.alpha. on peripheral blood mononuclear cells as shown by Scatchard anal. We addnl. show rsTNFR-p55.gamma.3 at high concns. also blocks T cell activation by dendritic cells. In conclusions rsTNFR-p55h.gamma.3 has a much higher anti-inflammatory effect than immunosuppressive effect.

L33 ANSWER 4 OF 12 MEDLINE

DUPLICATE 3

L33 ANSWER 2 OF 12

MEDLINE

DUPLICATE 2

ACCESSION NUMBER: 97163923 MEDLINE
DOCUMENT NUMBER: 97163923 PubMed ID: 9010678
TITLE: Production of prostaglandin E2 and collagenase is inhibited

by the recombinant soluble **tumour necrosis factor** receptor p55-human **gamma** 3 fusion protein at concentrations a hundred-fold lower than those decreasing T cell

activation.

AUTHOR: Nicod L P; Isler P; Chicheportiche R; Songeon F; Dayer J M
CORPORATE SOURCE: Respiratory Division, University Hospital, Geneva, Switzerland.

SOURCE: EUROPEAN CYTOKINE NETWORK, (1996 Dec) 7 (4) 757-63.

Journal code: 9100879. ISSN: 1148-5493.

PUB. COUNTRY: France

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199703

ENTRY DATE: Entered STN: 19970407

Last Updated on STN: 19970407

Entered Medline: 19970326

AB TNF-alpha and lymphotoxin alpha (TNF-beta) are pleiotropic cytokines with regulatory functions in inflammatory reactions and T cell activation. Natural TNF inhibitors such as soluble TNF-binding proteins, i.e.

TNFsR55

and TNFsR75, are shed from white blood cells and probably other cells. These naturally occurring inhibitors of TNF are shown to be 10 times less effective than the bivalent antagonist of TNF, recombinant soluble TNF receptor p55-human gamma 3 fusion protein (rsTNFR-p55h gamma 3), in controlling the release of prostaglandin E2 (PGE2) and collagenase by fibroblasts, as well as in controlling **T cell proliferation**. In order to block the action of rhTNF-alpha added to fibroblasts, a fivefold excess of rsTNFR-p55h gamma 3 was sufficient, but concentrations of a hundred to a thousand times higher were required to obtain a significant inhibition of T cell activation. This concentration appears to be required to block membrane-bound TNF-alpha on peripheral blood mononuclear cells as shown by Scatchard analysis. We additionally show that rsTNFR-p55h gamma 3 at high concentrations also blocks T cell activation by dendritic cells. In conclusion rsTNFR-p55h gamma 3 has a much higher anti-inflammatory effect than immunosuppressive effect.

L38 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2000:790340 CAPLUS

DOCUMENT NUMBER: 133:355211

TITLE: Death domain-contg. receptor 5 and compns. for treatment of immunity-related diseases, viral diseases, and cancer

INVENTOR(S): Ni, Jian; Gentz, Reiner L.; Yu, Guo-liang; Rosen, Craig A.

PATENT ASSIGNEE(S): Human Genome Sciences, Inc., USA

SOURCE: PCT Int. Appl., 266 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000066156	A1	20001109	WO 2000-US12041	20000504
W:			AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM	
RW:			GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG	
EP 1196191	A1	20020417	EP 2000-930329	20000504
R:			AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO	
JP 2002543151	T2	20021217	JP 2000-615040	20000504
US 2002072091	A1	20020613	US 2001-874138	20010606

PRIORITY APPLN. INFO.:

US 1999-132498P	P	19990504
US 1999-133238P	P	19990507
US 1999-148939P	P	19990813
US 1997-40846P	P	19970317
US 1997-54021P	P	19970729
US 1998-42583	A1	19980317
US 2000-565009	A1	20000504
WO 2000-US12041	W	20000504

AB The present invention relates to novel Death Domain Contg. Receptor-5 (DR5) proteins which are members of the tumor necrosis factor (TNF) receptor family, and have now been shown to bind TRAIL. In particular, isolated nucleic acid mols. are provided encoding the human DR5 proteins. DR5 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates

to screening methods for identifying agonists and antagonists of DR5 activity, e.g., for treating graft-vs.-host disease, viral infection, cancer, and immune diseases.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

Good data

L78 ANSWER 17 OF 21

MEDLINE

DUPLICATE 2

ACCESSION NUMBER: 97086827 MEDLINE
DOCUMENT NUMBER: 97086827 PubMed ID: 8932856
TITLE: Interferon-gamma plays a key role in the human mixed lymphocyte culture.
AUTHOR: Danzer S G; aCampo C; Rink L
CORPORATE SOURCE: Institute of Immunology and Transfusion Medicine, University of Lubeck School of Medicine, Germany.
SOURCE: BONE MARROW TRANSPLANTATION, (1996 Nov) 18 (5) 991-6.
Journal code: 8702459. ISSN: 0268-3369.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970306
Last Updated on STN: 19970306
Entered Medline: 19970225

AB Measurement of cytokines in the mixed lymphocyte culture (MLC) is thought to be a new and relevant parameter for bone marrow transplantation (BMT). Our experiments showed that IFN-gamma plays a central role in the cytokine network following alloantigenic recognition. IFN-gamma itself is induced by IL-2 since anti-IL-2 strongly reduced the secretion of IFN-gamma. As anti-IFN-gamma also diminished the response of IL-2 and sIL-2R, a feedback mechanism between these two cytokines is assumed. Addition of rIFN-gamma to the MLC augmented the release of sCD8 molecules, whereas sCD4 molecules were reduced, indicating that IFN-gamma led to T cell differentiation instead of IL-2 dependent proliferation. In the MLC, a feedback mechanism between TNF-alpha and IFN-gamma exists, since **anti-TNF-gamma** reduced the secretion of IFN-gamma and anti-IFN-gamma inhibited the release of TNF-alpha. Therefore, IFN-gamma plays a critical role in monocyte activation, T cell differentiation, and IL-2-induced cell growth. We conclude that measurement of IFN-gamma might be a new and more sensitive parameter for BMT than the established proliferation assay, since IFN-gamma directly quantifies T cell activation.

L78 ANSWER 7 OF 21 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

ACCESSION NUMBER: 2001-13023 BIOTECHDS

TITLE: Humanization of a mouse monoclonal **antibody**
neutralizing **TNF-gamma** by guided
selection;
plasmid p3MH expression in Escherichia coli, hybridoma
cell culture, phage display

AUTHOR: Wang Z; Wang Y; Li Z; Li J; Dong Z

CORPORATE SOURCE: Navy-Gen.Hosp.Beijing; Univ.Beijing-Med.;
Acad.Med.Sci.Beijing

LOCATION: Central Laboratory, Navy General Hospital, Haidian District,
Beijing 100037, People's Republic of China.

Email: zwangl@partners.org

SOURCE: J.Immunol.Methods; (2001) 241, 1-2, 171-84

CODEN: JIMMBG

ISSN: 0022-1759

DOCUMENT TYPE: Journal

LANGUAGE: English

AN 2001-13023 BIOTECHDS

AB Humanization of a mouse monoclonal **antibody** neutralizing tumor
necrosis factor (**TNF**)-**gamma** by guided selection was
discussed. Human recombinant and the hybridoma Z8, which produces a
mouse monoclonal **anti-TNF-gamma**
antibody of **immunoglobulin** (Ig)G1 subclass with the k
light chain were used. The isolated Fd and human k genes emerged from
guided selection were recloned into the expression vector p3MH and the
recombinant vectors were transformed to Escherichia coli TG-1. One of
the isolated human Fd genes (huFd2), which showed the strongest
reactivity, was chosen to pair with 12 of selected human k chains. Two
of the resulting human Fabs (huFad2-huk1 and huFad2-huk2), with same Fd
and different k chains, bound to **TNF-gamma** specificity. Their human
origin was proved by ELISA and sequencing analysis. The human Fabs
competitive ELISA and in vitro **TNF-gamma** neutralization assay showed

that

the human Fabs resembled its parental mouse mAb Z8 in that they both
recognized the same epitope and neutralized the cytotoxicity of
TNF-gamma. (32 ref)

(FILE 'HOME' ENTERED AT 19:32:50 ON 13 MAY 2003)

FILE 'MEDLINE, BIOSIS, CANCERLIT, LIFESCI, CAPLUS, BIOTECHDS' ENTERED AT 19:33:07 ON 13 MAY 2003

L1 14 S TNFG OR GTNF OR TNFGAMMA OR GAMMATNF
L2 3256 S TNF(A) (G OR GAMMA)
L3 251510 S (TUMOR OR TUMOUR) (W) NECROSIS (W) (FACTOR)
L4 17384 S L3 (3A) (G OR GAMMA OR FIBROBLAST)
L5 0 S TNF(A) FIBROBLAST
L6 0 S TNF(3A) FIBROBLAST
L7 19840 S L1 OR L2 OR L4
L8 1560 S L7 (5A) (NEUTROPHIL? OR MACROPHAGE# OR MONOCYT?)
L9 17 S L8 (5A) (PROLIFER?)
L10 10 DUP REM L9 (7 DUPLICATES REMOVED)
L11 1274 S L7 (5A) FIBROBLAST#
L12 875 S L11 AND PY<1998
L13 36 S L11 (5A) (PROLIF? (A) FIBROBLAST?)
L14 47 S L11 (5A) (PROLIF? (3A) FIBROBLAST?)
L15 26 S L14 AND PY<1998
L16 22 DUP REM L15 (4 DUPLICATES REMOVED)

FILE 'MEDLINE' ENTERED AT 20:05:24 ON 13 MAY 2003

L17 3 S TNFG OR GTNF OR TNFGAMMA OR GAMMATNF
L18 671 S TNF(A) (G OR GAMMA)
L19 54995 S (TUMOR OR TUMOUR) (W) NECROSIS (W) (FACTOR)
L20 3502 S L19 (3A) (G OR GAMMA OR FIBROBLAST)
L21 4023 S L17 OR L18 OR L20
L22 7 S L21 (S) (PROLIF? (3A) FIBROBLAST#)
L23 650 S L21 (S) (FIBROBLAST# OR (T(W) (CELL# OR LYMPHOCYTE#)))

FILE 'MEDLINE, BIOSIS, CANCERLIT, LIFESCI, BIOTECHDS, CAPLUS' ENTERED AT 20:11:19 ON 13 MAY 2003

L24 16242 S L3 (3A) (G OR GAMMA)
L25 18700 S L1 OR L2 OR L24
L26 54 S L25 (S) (PROLIF? (3A) (FIBROBLAST# OR NEUTROPHIL# OR MONOCYTE# O
L27 38 S L26 AND PY<1998
L28 19 DUP REM L27 (19 DUPLICATES REMOVED)
L29 3123 S L25/TI
L30 72911 S PROLIFER? (3A) (FIBROBLAST# OR NEUTROPHIL? OR MONOCYTE# OR
(T(W)
L31 60 S L29 AND L30
L32 50 S L31 AND PY<1998
L33 12 DUP REM L32 (38 DUPLICATES REMOVED)
L34 238 S TNF(W) (G OR GAMMA)
L35 252 S (TUMOR OR TUMOUR) (W) NECROSIS (W) (FACTOR) (W) (G OR GAMMA)
L36 466 S L1 OR L34 OR L35
L37 19 S L36 AND L30
L38 11 DUP REM L37 (8 DUPLICATES REMOVED)

FILE 'PCTFULL, USPATFULL, EUROPATFULL' ENTERED AT 20:36:00 ON 13 MAY 2003

L39 705 S TNF(W) (G OR GAMMA)
L40 154 S (TUMOR OR TUMOUR) (W) NECROSIS (W) (FACTOR) (W) (G OR GAMMA)
L41 83 S TNFG OR GTNF OR TNFGAMMA OR GAMMATNF
L42 13681 S PROLIFER? (3A) (FIBROBLAST# OR NEUTROPHIL? OR MONOCYTE# OR
(T(W)
L43 879 S L39 OR L40 OR L41
L44 34 S L43/TI,AB

L45 7 S L44 AND L42
 L46 1 S L45 AND AD<19970212
 L47 79 S L43(S)L42
 L48 5 S L47 AND AD<19970212
 L49 2 S L48 AND PD>20001129
 L50 3 S L48 NOT L49
 L51 3491 S YU/IN
 L52 422 S NI/IN
 L53 57 S L51 AND L52
 L54 14 S L53 AND L43
 L55 1 S L54 AND AD<19970212
 L56 3 S L53 AND (RECEPTOR(W)5)/TI
 L57 97 S L43(5A)PROLIF?
 L58 3 S L57 AND AD<19970212
 L59 44599 S METASTA?
 L60 4491 S L59(S)ENDOTHELI?
 L61 529 S L60 AND L43
 L62 9 S L44 AND L60
 L63 0 S L62 AND AD<19970212
 L64 63 S L60(S)L43
 L65 0 S L64 AND AD<19970212
 L66 12 S L61 AND AD<19970212
 L67 95 S L60/TI,AB
 L68 0 S L67 AND L43
 L69 27 S L67 AND AD<19970212

FILE 'MEDLINE, BIOSIS, CANCERLIT, LIFESCI, BIOTECHDS, CAPLUS' ENTERED AT
 21:22:55 ON 13 MAY 2003

L70 8853 S METASTA?(S)ENDOTHELI?
 L71 0 S L70 AND L36
 L72 613410 S METASTA?
 L73 20 S L72 AND L36
 L74 16 DUP REM L73 (4 DUPLICATES REMOVED)
 L75 4 S ANTI(W)L36
 L76 20 S L36(5A)(ANTIBOD? OR IMMUNOGLOBULIN#)
 L77 23 S L75 OR L76
 L78 21 DUP REM L77 (2 DUPLICATES REMOVED)
 L79 0 S OSTEOCLAST# AND L36
 L80 1 S OSTEOBLAST# AND L36

FILE.'PCTFULL, USPATFULL, EUROPATFULL' ENTERED AT 21:42:44 ON 13 MAY 2003

L81 0 S L44 AND (OSEO?)
 L82 17 S L44 AND OSTEO?
 L83 2 S L82 AND AD<19970212

L69 ANSWER 19 OF 27 USPATFULL

ACCESSION NUMBER: 1998:72589 USPATFULL

TITLE: Heparin- and sulfatide binding peptides from the type I

INVENTOR(S): repeats of human thrombospondin and conjugates thereof
Roberts, David D., Bethesda, MD, United States
Browning, Philip J., Brentwood, TN, United States
Bryant, Joseph L., Bethesda, MD, United States
Inman, John K., Bethesda, MD, United States
Krutzsch, Henry C., Bethesda, MD, United States
Guo, Nenghua, Gaithersburg, MD, United States

PATENT ASSIGNEE(S): The United States of America as represented by the
Department of Health and Human Services, Washington,
DC, United States (U.S. government)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5770563		19980623
APPLICATION INFO.:	US 1995-487568		19950607 (8) <--
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 1994-215085, filed on 21 Mar 1994, now abandoned which is a continuation-in-part of Ser. No. US 1991-801812, filed on 6 Dec 1991, now patented, Pat. No. US 5357041		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Tsang, Cecilia J.		
ASSISTANT EXAMINER:	Harle, Jennifer		
LEGAL REPRESENTATIVE:	Townsend and Townsend and Crew, LLP		
NUMBER OF CLAIMS:	41		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	64 Drawing Figure(s); 63 Drawing Page(s)		
LINE COUNT:	3518		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AI US 1995-487568 19950607 (8) <--
AB . . . motility, extravasation and neovascularization), sulfatides,
related sulfated glycoconjugates, fibronectin, and basic fibroblast
growth factor, involving malignant cell lines and normal
endothelial cells. Use of the defined peptides, analogs or
peptidomimetics and their conjugates for treatment of **metastatic**
tumors, breast carcinomas, melanomas, Kaposi's sarcomas, hemangiomas,
diabetic retinopathies, and various pathological conditions dependent
upon neovascularization is also disclosed.

L69 ANSWER 20 OF 27 USPATFULL

L69 ANSWER 25 OF 27 USPATFULL

ACCESSION NUMBER: 92:3571 USPATFULL

TITLE: Cloned genes which encode ELAM-1

INVENTOR(S): Bevilacqua, Michael P., Holbrook, MA, United States
Gimbrone, Michael A., Jamaica Plain, MA, United States
Seed, Brian, Boston, MA, United States
Stengelin, Siegfried, Hofheim, Germany, Federal
Republic of

PATENT ASSIGNEE(S): Brigham & Women's Hospital, Boston, MA, United States
(U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5081034		19920114	
APPLICATION INFO.:	US 1988-270873		19881114	(7) <--
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	Granted			
PRIMARY EXAMINER:	Stone, Jacqueline			
ASSISTANT EXAMINER:	Low, Christopher S. F.			
LEGAL REPRESENTATIVE:	Sterne, Kessler, Goldstein & Fox			
NUMBER OF CLAIMS:	3			
EXEMPLARY CLAIM:	1			
NUMBER OF DRAWINGS:	2 Drawing Figure(s); 2 Drawing Page(s)			
LINE COUNT:	717			

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AI US 1988-270873 19881114 (7) <--

AB The invention relates to cloned genes, or degenerate variants thereof, which encode **endothelial**-leukocyte adhesion molecule-1 (ELAM-1), or fragments thereof. Such fragments may be leukocyte or complement-binding. The invention also relates to cloned genes. . . also relates to methods for the treatment of inflammation, post reperfusion injury, bacterial infections, vasculitis, leukemia, and methods of inhibiting **metastatic** spread of tumor cells by administering the pharmaceutical compositions of the invention.

ACCESSION NUMBER: 749981 EUROPATFULL EW 200303 FS PS
 TITLE: MONOCLONAL ANTIBODY SPECIFIC FOR PD-ECGF / THYMIDINE
 PHOSPHORYLASE.
 PD-ECGF / THYMIDIN PHOSPHORYLASE SPEZIFISCHER
 MONOKLONALER ANTIKOERPER.
 ANTICORPS MONOCLONAL SPECIFIQUE DU PD-ECGF / THYMIDINE
 PHOSPHORYLASE.
 INVENTOR(S): MIYADERA, Kazutaka, 29-8, Uearatacho, Kagoshima-shi,
 Kagoshima 890, JP;
 YAMADA, Yuji, 2-2953-21, Higashisayamagaoka,
 Tokorozawa-shi, Saitama 359, JP;
 TAKEBAYASHI, Yuji, 5959-24, Kamifukumotocho,
 Kagoshima-shi, Kagoshima 891-01, JP;
 AKIYAMA, Shin-ichi, 3-23-12, Koutokujidai,
 Kagoshima-shi, Kagoshima 891-01, JP
 PATENT ASSIGNEE(S): TAIHO PHARMACEUTICAL CO., LTD., 1-27, Kandanishiki-cho,
 Chiyoda-ku, Tokyo 101-0054, JP
 PATENT ASSIGNEE NO: 332997
 AGENT: Waechtershaeuser, Guenter, Prof. Dr., Patentanwalt, Tal
 29, 80331 Muenchen, DE
 AGENT NUMBER: 12711
 OTHER SOURCE: MEPB2003003 EP 0749981 B1 0016
 SOURCE: Wila-EPS-2003-H03-T1
 DOCUMENT TYPE: Patent
 LANGUAGE: Anmeldung in Japanisch; Veroeffentlichung in Englisch;
 Verfahren in Englisch
 DESIGNATED STATES: R AT; R BE; R CH; R DE; R DK; R ES; R FR; R GB; R GR; R
 IE; R IT; R LI; R LU; R MC; R NL; R PT; R SE
 PATENT INFO.PUB.TYPE: EPB1 EUROPAEISCHE PATENTSCHRIFT (Internationale
 Anmeldung)
 PATENT INFORMATION:

	PATENT NO	KIND	DATE
	EP 749981	B1	20030115
'OFFENLEGUNGS' DATE:			19961227
APPLICATION INFO.:	EP 1995-941870		19951225
PRIORITY APPLN. INFO.:	JP 1994-327328		19941228
RELATED DOC. INFO.:	WO 95-JP2661	951225	INTAKZ
	WO 96020217	960704	INTPNR
REFERENCE PAT. INFO.:	EP 377855 A		JP 2288897 A
REF. NON-PATENT-LIT.:	ASAI K ET AL: "Neurotrophic action of gliostatin on cortical neurons. Identity of gliostatin and platelet - derived endothelial cell growth factor." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 267, no. 28, 5 October 1992 (1992-10-05), pages 20311-6, XP002112454 TOI M ET AL: "Expression of platelet - derived endothelial cell growth factor / thymidine phosphorylase in human breast cancer." INTERNATIONAL JOURNAL OF CANCER, vol. 64, no. 2, 21 April 1995 (1995-04-21), pages 79-82, XP002112676 MOGHADDAM A. ET AL: "Thymidine phosphorylase is angiogenic and promotes tumor growth." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 92, no. 4, February 1995 (1995-02), pages 998-1002, XP002112455 TAKEBAYASHI Y ET AL: "Clinicopathological significance of an angiogenic enzyme, thymidine phosphorylase (dThdPase), in		

colorectal carcinoma (Meeting abstract)." PROC ANNU
MEET AM ASSOC CANCER RES, vol. 36, March 1995 (1995-03),
page A535 XP002112456 OGAWA Y ET AL: "An evaluation of
thymidine phosphorylase (Dthd Pase) in breast cancer
with immunohistochemical staining (Meeting abstract)."
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(1995-03), page A544 XP002112457 J. BIOCHEM., (1993),
Vol. 114, No. 1, SUMIZAWA, T. et al., "Thymidine
Phosphorylase Activity Associated with Platelet-Derived
Endothelial Cell Growth Factor", p. 9-14. CANCER
RESEARCH, (1993), Vol. 53, No. 23, HARAGUCHI, M. et
al.,
" Sensitivity of Human KB Cells Expressing
Platelet-Derived Endothelial Cell Growth Factor to
Pyrimidine Antimetabolites", p. 5680-5682. OSAMU
KANEMITSU, "Introduction of Antibody", January 25,
1994,
CHIJINSHOKAN, p. 75-144. NATURE, (1992), Vol. 356,
FURUKAWA, T. et al., "Angiogenic Factor", p. 668.
NATURE, (1989), Vol. 338, ISHIKAWA, F. et al.,
"Identification of Angiogenic Activity and the Cloning
and Expression of Platelet-Derived Endothelial Cell
Growth Factor", p. 557-562

AI EP 1995-941870 19951225
AI EP 1995-941870 19951225
ABEN A monoclonal antibody against a peptide which occurs in human thymidine
phosphorylase and vascular **endothelial** cell growth factor
originating in human platelets and contains amino acid sequences of SEQ
ID NOS: 1 and 2; and a method of immunoassay of human thymidine
phosphorylase and/or vascular **endothelial** cell growth factor
originating in human platelet by using this monoclonal antibody. The
monoclonal antibody recognizes human thymidine phosphorylase and
vascular **endothelial** cell growth factor originating in human
platelet and is useful in the diagnosis and treatment of various
tumors,
metastasis thereof, diseases accompanied by abnormal
vascularization, etc.

L16 ANSWER 19 OF 22 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1989:93274 CAPLUS

DOCUMENT NUMBER: 110:93274

TITLE: Suppression of TNF-stimulated proliferation of
diploid

fibroblasts and TNF-induced cytotoxicity against
transformed fibroblasts by TGF-.beta.

AUTHOR(S): Kamiyo, Ryutaro; Takeda, Ken; Nagumo, Masao; Konno,
Kunio

CORPORATE SOURCE: Sch. Med., Showa Univ., Tokyo, 142, Japan

SOURCE: Biochemical and Biophysical Research Communications (
1989), 158(1), 155-62

CODEN: BBRCA9; ISSN: 0006-291X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Human transforming growth factor-.beta. (TGF-.beta.) concn.-dependently
inhibited proliferation of WI-38 cells, normal human diploid fibroblasts,
stimulated by tumor necrosis factor (TNF). Inhibition occurred at 1
ng/mL

concn. of TGF-.beta.. Also, TGF-.beta. dose-dependently suppressed
cytotoxicity of TNF against L-929 cells, murine transformed fibroblasts.
The concn. of TNF required for 50% cytolysis of L-929 cells was changed
from 30 ng/mL to 350 ng/mL by 10 ng/mL TGF-.beta.. This suppression was
abolished when L-929 cells were treated with actinomycin D or
cycloheximide, suggesting that TGF-.beta. might inhibit the action of TNF
via de novo protein synthesis. This response was not due to down
regulation of TNF receptor nor to alteration of the affinity of TNF for
its receptor.

L16 ANSWER 20 OF 22 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1989:405618 CAPLUS

DOCUMENT NUMBER: 111:5618

TITLE: Modulation of mitogenic activity of tumor necrosis factor by interferons and dexamethasone

AUTHOR(S): Tsujimoto, Masafumi; Sugiyama, Masako; Adachi, Hideki

CORPORATE SOURCE: Suntory Inst. Biomed. Res., Mishima, 618, Japan

SOURCE: Lymphokine Research (1989), 8(2), 99-106

CODEN: LYREDH; ISSN: 0277-6766

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Effect of interferon (IFN) on fibroblast growth enhancing activity of tumor necrosis factor (TNF) was examd. IFN-.gamma. and -.beta. inhibited TNF-mediated growth stimulation of FS-4 cells. IFNs also inhibited dexamethasone (DEX)-mediated amplification of mitogenic activity of TNF. Significant inhibition was still demonstrable when IFN-.gamma. was added

2

days after treatment with TNF. On the other hand, no mitogenic activity of TNF was obsd. when cells were pretreated with IFN-.gamma. for 6 h. These results suggested that interaction between TNF and IFN-.gamma.

might

play a role in modulation of some inflammatory processes in vivo.

L38 ANSWER 9 OF 11

MEDLINE

DUPLICATE 1

ACCESSION NUMBER: 96264103 MEDLINE

DOCUMENT NUMBER: 96264103 PubMed ID: 8666424

TITLE: Effect of human cytokines (IFN-gamma, TNF-alpha, IL-1 beta,

IL-4) on porcine endothelial cells: induction of MHC and adhesion molecules and functional significance of these changes.

AUTHOR: Batten P; Yacoub M H; Rose M L

CORPORATE SOURCE: Heart Science Centre, National Heart and Lung Institute at Harefield Hospital, Middlesex, UK.

SOURCE: IMMUNOLOGY, (1996 Jan) 87 (1) 127-33.
Journal code: 0374672. ISSN: 0019-2805.

PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199608

ENTRY DATE: Entered STN: 19960819

Last Updated on STN: 19970203

Entered Medline: 19960807

AB Previous studies using cultured human endothelial cells have demonstrated the role of inflammatory cytokines [interferon-gamma (IFN-gamma), **tumour necrosis factor-gamma** (TNF-alpha), interleukin-1 beta (IL-1 beta) and IL-4] in the regulation

of

major histocompatibility complex (MHC) and adhesion molecule expression. These cytokines are therefore implicated in the amplification of allograft, and more recently xenograft, rejection. In view of the likely event of grafted porcine tissues being exposed to human cytokines, we

have

investigated the effect of IFN-gamma, TNF-alpha, IL-1 beta, IL-4 and recombinant porcine IFN-gamma (rpoIFN-gamma) on cultured porcine aortic endothelial cells (PAEC) with respect to induction/up-regulation of porcine MHC and adhesion molecules and B7 receptors. Expression was detected using monoclonal antibodies (mAb) against porcine ligands and human CTLA-4-immunoglobulin; binding was analysed by flow microfluorimetry. TNF-alpha but not the other human cytokines

unregulated

swine leucocyte antigens (SLA) class I, class II and B7 receptor expression and induced vascular cell adhesion molecule (VCAM) and E-selectin expression. Porcine IFN-gamma also up-regulated SLA class I and class II, the ligand for CTLA-4-immunoglobulin and VCAM expression; the magnitude and kinetics of this response differed to that produced by recombinant human TNF-alpha (rhTNF-alpha). The ability of untreated, rpoIFN-gamma- and rhTNF-alpha-treated PAEC to stimulate CD4+ T cell was compared. CD4+ **T-cell proliferation** and IL-2 production were significantly enhanced by rhTNF-alpha and rpoIFN-gamma, rpoIFN-gamma being more effective than rhTNF-alpha. Use of blocking antibodies and CTLA-4-immunoglobulin demonstrated that the enhanced proliferative response, but not apparently IL-2 production, was dependent on cytokine-mediated up-regulation of SLA class II and B7 receptors. In conclusion, human TNF-alpha acts as a proinflammatory cytokine on PAEC and is likely to enhance the cellular response to xenogeneic organs in vivo.

L16 ANSWER 22 OF 22 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1987:67178 BIOSIS

DOCUMENT NUMBER: BA83:35504

TITLE: SUPPRESSION OF BOTH MACROPHAGE-MEDIATED TUMOR CELL LYSIS AND CYTOLYTIC FACTOR PRODUCTION BY A FACTOR CYTOLYTIC INHIBITORY FACTOR DERIVED FROM NORMAL EMBRYONIC FIBROBLASTS.

AUTHOR(S): GALLILY R; GIFFORD G E; LOEWENSTEIN J

CORPORATE SOURCE: LAUTENBERG CENTER GENERAL TUMOR IMMUNOLOGY, HEBREW-UNIV. HADASSAH MED. SCH., JERUSALEM 91010, ISR.

SOURCE: CANCER IMMUNOL IMMUNOTHER, (1986) 23 (1), 60-66.
CODEN: CIIMDN. ISSN: 0340-7004.

FILE SEGMENT: BA; OLD

LANGUAGE: English

AB We had previously established a murine bone marrow-derived cell line, designated JBM.PHI.1.1, which displayed properties of normal macrophages, including the ability to perform macrophage-mediated cytotoxicity. It was also found that these cells could be induced by lipopolysaccharide (LPS) to produce reproducibly high levels of a cytotoxic factor (CF) resembling tumor necrosis factor (TNF). This cell line was therefore selected for further studies on macrophage-mediated tumor cell lysis and CF production.

Moreover, the CF production during incubation with LPS was higher in the absence of serum than in its presence, with a maximum at days 2-3 following the addition of LPS. A factor inhibitory to CF production (CIF) was detected in our laboratory in the supernatant of embryonic fibroblast cultures. We established the experimental conditions required for the optimal production and suppressive effect of CIF. High levels of CIF activity were obtained under conditions that promote fibroblast proliferation. Addition of embryonic fibroblast culture supernatant to the

macrophages shortly before LPS suppressed both LPS-induced CF production and tumoricidal activity. CIF did not affect macrophage protein synthesis in the presence or absence of LPS. However, LPS-induced interleukin 1 release was partially (55%) suppressed by embryonic fibroblast culture supernatant. Our results show that CIF does not exert a general inactivating effect on the macrophages, although it may possibly affect other functions in addition to CF production and tumor cell lysis. The strong inhibition of both the latter properties further indicates that TNF-like CF is an important mediator in macrophage-mediated tumor cell lysis.

L16 ANSWER 11 OF 22

MEDLINE

DUPLICATE 1

ACCESSION NUMBER: 91128413 MEDLINE

DOCUMENT NUMBER: 91128413 PubMed ID: 1993070

TITLE: Prostaglandins antagonize **fibroblast proliferation** stimulated by **tumor necrosis factor**.

AUTHOR: Hori T; Yamanaka Y; Hayakawa M; Shibamoto S; Tsujimoto M; Oku N; Ito F

CORPORATE SOURCE: Department of Biochemistry, Faculty of Pharmaceutical Sciences, Setsunan University, Osaka, Japan.

SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1991 Jan 31) 174 (2) 758-66.
Journal code: 0372516. ISSN: 0006-291X.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199103

ENTRY DATE: Entered STN: 19910405

Last Updated on STN: 19970203

Entered Medline: 19910308

AB Tumor necrosis factor (TNF) is known to be a mitogen for human diploid FS-4 fibroblasts. We have shown in an earlier study (Hori et al. (1989) J. Cell. Physiol. 141, 275-280) that indomethacin further enhances the cell proliferation stimulated by TNF. Since indomethacin inhibits the activity of cyclooxygenase, the role of prostaglandins in TNF-stimulated cell growth was examined. Cell growth stimulated by TNF and indomethacin was inhibited by exogenously added prostaglandins (PGE2, PGF2 alpha, and PGD2), among which PGE2 caused the greatest inhibition of cell growth. Treatment of FS-4 cells with 10 ng/ml TNF resulted in the release of prostaglandins (PGE2, 6-keto-PGF1 alpha, PGA2, PGD2, and PGF2 alpha) 2 to 4 fold over that of untreated cells. The amount of all these prostaglandins increased in a time-dependent manner over 6 h after treatment. In both TNF-treated and control cells, PGE2 was released as the predominant prostaglandin. Furthermore, when PGE2 production and DNA synthesis were determined in FS-4 cells treated with increasing doses of indomethacin, these two cellular responses were inversely affected by indomethacin. These data show that prostaglandins induced by TNF antagonize growth stimulatory action of TNF.

L16 ANSWER 5 OF 22 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1995:847142 CAPLUS

DOCUMENT NUMBER: 124:199946

TITLE: Transfection of cells with basic fibroblast growth factor and Kaposi fibroblast growth factor genes induce resistance to and receptor modulation of tumor necrosis factor

AUTHOR(S): Aggarwal, Bharat B.; Pocsik, Eva; Totpal, Klara
CORPORATE SOURCE: Cytokine Research Laboratory, Department of Molecular Oncology, The University of Texas M.D. Anderson

Cancer

SOURCE: Center, 1515 Holcombe Blvd., Houston, TX, 77030, USA
FEBS Letters (1995), 372(1), 44-8
CODEN: FEBLAL; ISSN: 0014-5793

PUBLISHER: Elsevier

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Tumor necrosis factor (TNF) has been shown to inhibit the growth of some cell types and stimulate the proliferation of others by a mechanism that is not understood. In the present study, the authors investigated the effect of transfection of NIH-3T3 cells with either the basic fibroblast growth factor gene (bFGF) or the Kaposi FGF gene (K-fgf) on the growth-modulatory effects of TNF. The results show that transformation

of

cells with either gene leads to resistance to the growth-inhibitory effects of TNF. The K-fgf gene was a more potent inducer of cellular resistance than the bFGF gene. The cellular resistance correlated with the inhibition of TNF-induced activation of phospholipase A2 and down-modulation of TNF receptors. Overall, the results indicate that

both

K-fgf and bFGF play an important role in suppression of antiproliferative effects of TNF.

L10 ANSWER 10 OF 10 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1990:75020 CAPLUS

DOCUMENT NUMBER: 112:75020

TITLE: Stimulation of polymorphonuclear neutrophils by TNF
(tumor necrosis factor) muteins

AUTHOR(S): Kamijo, Ryutaro; Takeda, Ken; Konno, Kunio; Nagumo,
Masao; Hasegawa, Akira; Inaka, Koji; Ikehara, Morio

CORPORATE SOURCE: Sch. Med., Showa Univ., Japan

SOURCE: Ensho (1989), 9(1), 25-8

CODEN: ENSHEE; ISSN: 0389-4290

DOCUMENT TYPE: Journal

LANGUAGE: Japanese

AB Tumor necrosis factor (TNF) plays an important role in inflammatory responses other than inducing hemorrhagic necrosis of animal tumors or exhibiting cytotoxicity to tumor cells. Five recombinant human TNF muteins (amino acid sequence partly changed by protein engineering techniques) were prepd. and their biol. activity in stimulating polymorphonuclear neutrophil functions were compared by measuring iodination activity. TNF (C-Phe) (leucine changed to phenylalanine in C-terminal) was more potent than the parent TNF in activating polymorphonuclear neutrophils, although the binding activity of TNF (C-Phe) to neutrophil membrane receptor was less than that of the parent TNF. Other TNF muteins also showed this activity in parallel with their receptor binding activity to neutrophils. The stimulating activity of

TNF muteins on polymorphonuclear function was proportional to the proliferation-enhancing activity on fibroblasts.

ACCESSION NUMBER: 522606 EUROPATFULL EW 199614 FS PS
 TITLE: Pyridine derivatives, their production and use.
 Pyridinderivate, deren Herstellung und Anwendung.
 Derives de pyridine, leur preparation et utilisation.
 INVENTOR(S): Takatani, Muneo, 6-1 Taniguchisono-machi, Ukyo-ku,
 Kyoto 616, JP;
 Saijo, Taketoshi, 5-9 Fushiodai 2-chome, Ikeda, Osaka
 563, JP;
 Tomimatsu, Kiminori, E-104, 7 Nyoidani 3-chome, Minoo,
 Osaka 562, JP
 PATENT ASSIGNEE(S): TAKEDA CHEMICAL INDUSTRIES, LTD., 1-1, Doshomachi
 4-chome, Chuo-ku, Osaka 541, JP
 PATENT ASSIGNEE NO: 204706
 AGENT: von Kreisler, Alek, Dipl.-Chem. et al, Patentanwaelte
 von Kreisler-Selting-Werner Postfach 10 22 41, D-50462
 Koeln, DE
 AGENT NUMBER: 12434
 OTHER SOURCE: EPB1996023 EP 0522606 B1 960403
 SOURCE: Wila-EPS-1996-H14-T1
 DOCUMENT TYPE: Patent
 LANGUAGE: Anmeldung in Englisch; Veroeffentlichung in Englisch
 DESIGNATED STATES: R AT; R BE; R CH; R DE; R DK; R ES; R FR; R GB; R GR; R
 IT; R LI; R LU; R NL; R PT; R SE
 PATENT INFO.PUB.TYPE: EPB1 EUROPAEISCHE PATENTSCHRIFT
 PATENT INFORMATION:

PATENT NO	KIND	DATE
EP 522606	B1	19960403
		19930113
EP 1992-201288		19920507
JP 1991-105691		19910510
EP 103503 A	EP 177907	A
US 3687959 A	US 4786644	A

'OFFENLEGUNGS' DATE: 1,
 APPLICATION INFO.: 6 July 1964, Columbus, Ohio, US; abstract no. 638g,
 PRIORITY APPLN. INFO.: I.KH. FEL DMAN ET AL. 'Synthesis in the pyridine
 REFERENCE PAT. INFO.: series.
 REF. NON-PATENT-LIT.: III. Synthesis of quaternary derivatives of
 beta-aminoethyl pyridyl sulfides'

L66 ANSWER 2 OF 12 PCTFULL COPYRIGHT 2003 Univentio
ACCESSION NUMBER: 1996001653 PCTFULL ED 20020514
TITLE (ENGLISH): METHODS AND COMPOSITIONS FOR THE SPECIFIC COAGULATION
OF VASCULATURE
TITLE (FRENCH): PROCEDES ET COMPOSITIONS POUR LA COAGULATION
SPECIFIQUE

INVENTOR(S): DE VAISSEAUX
THORPE, Philip, E.;
EDGINGTON, Thomas, S.
PATENT ASSIGNEE(S): BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM;
THE SCRIPPS RESEARCH INSTITUTE;
THORPE, Philip, E.;
EDGINGTON, Thomas, S.

LANGUAGE OF PUBL.: English

DOCUMENT TYPE: Patent

PATENT INFORMATION:

NUMBER	KIND	DATE
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WO 9601653	A1	19960125
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DESIGNATED STATES

W:

AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE
HU IS JP KE KG KP KR KZ LK LR LT LU LV MD MG MN MW MX
NO NZ PL PT RO RU SD SE SG SI SK TJ TT UA US UZ VN KE
MW SD SZ UG AT BE CH DE DK ES FR GB GR IE IT LU MC NL
PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

APPLICATION INFO.: WO 1995-US7439 A 19950607

PRIORITY INFO.: US 1994-8/273,567 19940711

AI WO 1995-US7439 A 19950607

DETD Exemplary inducible antigens include those inducible
by a cytokine, e.g., IL-1, IL-4, TNF-u, **TNF-g** or
IFN-T,
as may be released by monocytes, macrophages, mast cells,
helper T cells, CD8-positive T-cells, NK cells or even
tumor cells. Examples. . .

The induction of tumor infarction by targeting
coagulation-inducing proteins to these and other tumor
endothelial cell markers is proposed as a valuable new
approach to the treatment of solid tumors. The coupling
of human (or humanized) antibodies. . . wholly human coaguligands is
particularly contemplated, thus permitting repeated
courses of treatment to be given to combat both the
primary tumor and its **metastases**.

L55 ANSWER 1 OF 1 PCTFULL COPYRIGHT 2003 Univentio
 ACCESSION NUMBER: 1996014328 PCTFULL ED 20020514
 TITLE (ENGLISH): **TUMOR NECROSIS FACTOR-
 GAMMA**
 TITLE (FRENCH): POLYPEPTIDE GAMMA APPARTENANT A LA FAMILLE DES
 FACTEURS DE NECROSE TUMORALE (FNT)
 INVENTOR(S): **YU, Guo-Liang;**
NI, Jian;
 ROSEN, Craig, A.
 PATENT ASSIGNEE(S): HUMAN GENOME SCIENCES, INC.;
 YU, Guo-Liang;
 NI, Jian;
 ROSEN, Craig, A.
 LANGUAGE OF PUBL.: English
 DOCUMENT TYPE: Patent
 PATENT INFORMATION:

NUMBER	KIND	DATE

WO 9614328	A1	19960517

DESIGNATED STATES
 W: AU CA CN JP KR NZ US AT BE CH DE DK ES FR GB GR IE IT
 LU MC NL PT SE
 APPLICATION INFO.: **WO 1994-US12880 A 19941107**
 TIEN **TUMOR NECROSIS FACTOR-GAMMA**
 IN **YU, Guo-Liang;**
NI, Jian;
 ROSEN, Craig, A.

AI **WO 1994-US12880 A 19941107**
 ABEN A human **TNF-gamma** polypeptide and DNA (RNA) encoding
 such polypeptide and a procedure for
 producing such polypeptide by recombinant techniques is disclosed.
 Also.
 . . . certain cell types to treat diseases, for example restenosis.
 Also

disclosed are diagnostic methods for detecting a mutation in the
TNF-gamma nucleic acid sequence or
 an overexpression of the **TNF-gamma** polypeptide.
 Antagonists against such polypeptides and their use
 as a therapeutic to treat cachexia, septic shock, cerebral malaria,
 inflammation, arthritis. . .

DETD . . . has been
 identified as a novel member of the TNF family based on
 structural, amino acid sequence homology, and functional
 similarities, for example, **TNF-gamma** is a
 pro-inflammatory
 protein.

In accordance with one aspect of the present invention,
 there is provided a novel mature polypeptide which is **TNF-
 gamma**, as well as biologically active and diagnostically or
 therapeutically useful fragments, analogs and derivatives
 thereof. The polypeptide of the present invention is. . .

in accordance with another aspect of the present
 invention, there are provided isolated nucleic acid molecules

encoding human **TNF-gamma**, including mRNAs, DNAs, cDNAs, genomic DNAs as well as analogs and biologically active and diagnostically or therapeutically useful fragments and derivatives thereof.

there is provided a process for producing such polypeptide by recombinant techniques comprising culturing recombinant prokaryotic and/or eukaryotic host cells, containing a human **TNF-gamma** nucleic acid sequence, under conditions promoting expression of said protein and subsequent recovery of said protein.

present invention, there are also provided nucleic acid probes comprising nucleic acid molecules of sufficient length to specifically hybridize to human **TNF-gamma** sequences.

In accordance with another aspect of the present invention, there are provided **TNF-gamma** agonists which mimic

TNF-gamma and binds to the **TNF-gamma** receptors to elicit **TNF-gamma** type responses.

another aspect of the present invention, there are provided diagnostic assays for detecting diseases related to the under-expression and over-expression of the **TNF-gamma** polypeptide and nucleic acid sequences encoding such polypeptide.

Figure 2 illustrates an amino acid sequence alignment between **TNF-gamma** and other members of the TNF family. **TNF-gamma**

gamma contains the conserved amino acid residues of the TNF family as shown by the shaded areas.

Figure 3A is an RNA blot analysis showing the human tissues where **TNF-gamma** is expressed. RNA from the tissues indicated were probed with labeled **TNF-gamma** cDNA.

TNF-gamma

MRNA exists predominantly in the kidney since Figure 3A shows a distinct band. Other lanes seem to show strong hybridization, however, these are. . .

Figure 3B is an RNA blot analysis showing that **TNF-gamma**

is expressed predominantly in HUVEC cells (human umbilical vein endothelial cells) which is lane 9. Lane 6 and lane 8 are non-specific smears. RNA from the cell lines indicated were probed with labeled **TNF-gamma** cDNA. Lane 1 is CAMAI

(breast cancer) ; lane 2 AN3CA (uterine cancer) ; lane 3, SK.UT.1 (uterine cancer); lane 4, MG63. . .

Figure 4 is a photograph of a gel after electrophoresing **TNF-gamma** was produced by bacterial expression and purification.

Figure 5 is a photograph of a gel after baculovirus expression of **TNF-gamma**.

Figure 6B illustrates the ability of **TNF-gamma** in comparison to TNF-a and TNF-0 to inhibit WEHI 164 cell growth.

Figure 7 illustrates the ability of recombinant **TNF-gamma**, TNF-a and TNF-0 to induce WEHI 164 cell death.

Figure 8 illustrates the ability of recombinant TNF-a, TNF-fl, and **TNF-gamma** to induce morphological change in L929 cells. The morphology change is indicated by dark round cells. Cells were treated with E. Coli. . .

Figure 9 is a graphical illustration of the effect of **TNF-gamma**, TNF-a and TNF-0 on venous endothelial cells. Cell proliferation after venous endothelial cells were treated with commercially available TNF-ce and TNF-0 and E. Coli produced **TNF-gamma** was quantified using an MTS assay.

Figure 10 is a photograph of HL60 cells, with control showing the HL60 cells being spread apart; TNF-a and **TNF-gamma** induce cell adhesion and cell-cell contact as illustrated by the cells adhering together in the lower right.

Figure 11 illustrates that **TNF-gamma** does not significantly bind to two known soluble TNF receptors, namely sTNF RI (p55) and sTNF RII (p75).

Sequences conserved throughout the members of the TNF family are also conserved in **TNF-gamma** (see Figure 2). The bolded letters indicate conserved amino acid residues. The **TNF-gamma** mRNA is specifically expressed in human umbilical vein endothelial cells as shown in the RNA blot analysis of Figure 3B.

The present invention further relates to a **TNF-gamma** polypeptide which has the deduced amino acid sequence of Figure 1 or which has the amino acid sequence encoded by the deposited cDNA, . . .

etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the **TNF-gamma** genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, . . .

The **TNF-gamma** polypeptides can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation. . .

The **TNF-gamma** polypeptide of the present invention may be employed to inhibit tumor cell growth or neoplasia. The **TNF-gamma** polypeptide may be responsible for tumor destruction through apoptosis which is characterized by membrane blebbing (zeiosis) . condensation of cytoplasm and the activation of an endogenous endonuclease (Figure 7). As shown in Table 1. **TNF-gamma** has strong cytotoxic activity for the cell lines tested which have abnormal cellular proliferation and regulation, for example the fibrosarcoma and carcinoma cell line. This is also illustrated in Figure 6A, 6B and 8 where it is shown that **TNF-gamma** has the ability to inhibit L929 and WEHI 164 cell growth through cytotoxic activity. WEHI 164 cells are mouse fibrosarcoma cells. A preferable method of administering the **TNF-gamma** is by injection directly into the tumor.

The cell adhesion activity of **TNF-gamma** may be employed for wound healing. As shown in Table I and Figure 9, **TNF-gamma** has a strong endothelial cell proliferation effect which is an indication that **TNF-gamma** plays a role in wound healing. **TNF-gamma**'s cell adhesive effects may also play a role in wound healing.

TNF-gamma may also be employed to treat diseases which require growth promotion activity, for example, restenosis.

As stated above, **TNF-gamma** is shown to have strong proliferation effects on endothelial cell growth.

Accordingly, **TNF-gamma** may also be employed to regulate hematopoiesis and endothelial cell development.

The **TNF-gamma** polypeptide, through its ability to stimulate the activation of T-cells, is an important mediator of the immune response. Accordingly, this polypeptide may be used to stimulate an immune response against a variety of parasitic, bacterial and viral infections. **TNF-gamma** may lyse virus-infected cells and, therefore, be employed to arrest HIV infected cells.

The **TNF-gamma** polypeptide may also be employed to

treat autoimmune diseases such as Type I diabetes by enhancing the T-cell proliferative response.

Table 1

Summary of **TNF-gamma** activity
Cell lines Source and Activity
Type
Cyto- Prolif- Differ- Ad-
toxicity eration entiation hesion
L929 mouse fibroblast + -
WEHI 164 mouse
fibrosarcoma ... -
NRK-54E rat. . .

This invention provides a method for identification of the receptor for **TNF-gamma**. The gene encoding the receptor can be identified by numerous methods known to those of skill in the art, for example, ligand. . . Current Protocols in Immun., 1(2), Chapter St (1991)). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to **TNF-gamma**, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to **TNF-gamma**. Transfected cells which are grown on glass slides are exposed to labeled **TNF-gamma**. **TNF-gamma** can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase. Following. . .

As an alternative approach for receptor identification, labeled **TNF-gamma** can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE and exposed to X-ray film. The labeled complex containing the **TNF-gamma**-receptor can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to. . .

TNF-gamma does not bind significantly to two soluble TNF receptors, sTNF-RI (p55) and sTNF-RII (p75). Accordingly, **TNF-gamma** may have activities inclusive of and additional to known TNF proteins (see Figure 11).

This invention is also related to a method of screening compounds to identify those which mimic **TNF-gamma** (agonists) or prevent the effect of **TNF-gamma**. An example of such a method takes advantage of the ability of **TNF-gamma**

to significantly stimulate the proliferation of human endothelial cells in the presence of the mitogen Con A.

Alternatively, the response of a known second messenger system following interaction of **TNF-gamma** and receptor would be measured and compared in the presence or absence of the compound. Such second messenger systems include but are . . .

To assay for antagonists, the assay described above is performed, however, in this assay **TNF-gamma** is added along with the compound to be screened and the ability of the compound to inhibit NIThymidine incorporation in the presence of **TNF-gamma**, indicates that the compound is an antagonist to **TNF-gamma**. Alternatively, **TNF-gamma** antagonists may be detected by combining **TNF-gamma** and a potential antagonist with membrane-bound **TNF-gamma** receptors or recombinant receptors under appropriate conditions for a competitive inhibition assay. **TNF-gamma** can be labeled, such as by radioactivity, such that the number of **TNF-gamma** molecules bound to the receptor can determine the effectiveness of the potential antagonist.

Alternatively, a mammalian cell or membrane preparation expressing the **TNF-gamma** receptor is incubated with labeled **TNF-gamma** in the presence of the compound. The ability of the compound to enhance or block this. . .

Antibodies specific to **TNF-gamma** may be used as antagonists by binding to **TNF-gamma** and preventing it from binding to its receptor. Monoclonal antibodies are particularly effective in this regard. Antibodies specific to the **TNF-gamma** receptor, however, may mediate distinct cellular responses which tend to agonize the effects of **TNF-gamma** upon interaction with its receptor.

Potential **TNF-gamma** antagonists also include **TNF-gamma** mutants which bind to the **TNF-gamma** receptor and elicit no second messenger response to effectively block the receptor from its natural ligand. Specifically designed oligonucleotides and small molecules may also bind to the **TNF-gamma** receptor and block it from **TNF-gamma**. Examples of small molecules include but are not limited to small peptides or peptide-like molecules.

Another potential **TNF-gamma** antagonist is a soluble form of the **TNF-gamma** receptor which binds to **TNF-gamma** and prevents it from interacting with membrane-bound **TNF-gamma** receptors. In this way, the receptors are not stimulated by **TNF-gamma**.

Another potential **TNF-gamma** antagonist is an antisense construct prepared using antisense technology. Antisense technology can be used to control gene expression through triple-helix formation or antisense. . . al, Science, 241:456 (1988); and Dervan et al. , Science, 251: 1360 (1991)) , thereby preventing transcription and the production of **TNF-gamma**.

The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into the **TNF-gamma** polypeptide (Antisense - Okano,, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). The oligonucleotides. . . can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of **TNF-gamma**.

. . . employed to treat cachexia which is a lipid clearing defect resulting from a systemic deficiency of lipoprotein lipase which is suppressed by **TNF-gamma**. The **TNF-gamma** antagonists are also employed to treat cerebral malaria in which **TNF-gamma** appears to play a pathogenic role. The antagonists may also be employed to treat rheumatoid arthritis by inhibiting **TNF-gamma** induced production of inflammatory cytokines such as IL-1 in the synovial cells. When treating arthritis **TNF-gamma** is preferably injected intra-articularly.

The **TNF-gamma** antagonists may also be employed to prevent graft rejection by preventing the stimulation of the immune system in the presence of a graft by **TNF-gamma**.

The **TNF-gamma** antagonists may also be employed to treat osteoporosis since **TNF-gamma** may induce bone resorption. *osteoclasts?*

Antagonists to **TNF-gamma** may also be employed as anti-inflammation agents since **TNF-gamma** mediates an enhanced inflammatory response.

Fragments of the full length **TNF-gamma** gene may be used as a hybridization probe for a cDNA library to isolate the full length gene and to isolate other. . . be used to identify a cDNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete **TNF-gamma** gene including regulatory and promotor regions, exons, and introns. As an example of a screen comprises isolating the coding region of the **TNF-gamma** gene by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to that of the gene. . .

The **TNF-gamma** polypeptides and agonists and antagonists of the present invention may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a. . .

The **TNF-gamma** polypeptides and agonists and antagonists which are polypeptides may also be employed in accordance with the present invention by expression of such. . .

This invention is also related to the use of the **TNF-gamma** gene as part of a diagnostic assay for detecting diseases or susceptibility to diseases related to the presence of mutated **TNF-gamma**. Such diseases are related to an under-expression of **TNF-gamma**, for example, abnormal cellular proliferation such as tumors and cancers.

Individuals carrying mutations in the human **TNF-gamma** gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's. . .

As an example, PCR primers complementary to the nucleic acid encoding **TNF-gamma** can be used to identify and analyze **TNF-gamma** mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled **TNF-gamma** RNA or alternatively, radiolabeled **TNF-gamma** antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

The present invention also relates to a diagnostic assay for detecting altered levels of **TNF-gamma** protein in various tissues since an over-expression of the proteins compared to

normal control tissue samples may detect the presence of a disease or susceptibility to a disease, for example, tumors and cerebral malaria. Assays used to detect levels of **TNF-gamma** protein in a sample derived from a host are well-known to those of skill in the art and include radioimmunoassays, competitive-binding assays, . . . assay. An ELISA assay (Coligan, et al., Current Protocols in Immunology, 1(2), Chapter 6, (1991)) initially comprises preparing an antibody specific to the **TNF-gamma** antigen, preferably a monoclonal antibody. In addition a reporter antibody is prepared against the monoclonal antibody. To the reporter antibody is attached. . . .

Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any **TNF-gamma** proteins attached to the polystyrene dish. All unbound monoclonal antibody is washed out with buffer. The reporter antibody linked to horseradish peroxidase is now placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to **TNF-gamma**. Unattached reporter antibody is then washed out. Peroxidase substrates are then added to the dish and the amount of color developed in a given time period is a measurement of the amount of **TNF-gamma** protein present in a given volume of patient sample when compared against a standard curve.

A competition assay may be employed wherein antibodies specific to **TNF-gamma** are attached to a solid support and labeled **TNF-gamma** and a sample derived from the host are passed over the solid support and the amount of label detected, for example by liquid scintillation chromatography, can be correlated to a quantity of **TNF-gamma** in the sample.

A sandwich assay is similar to an ELISA assay. In a sandwich assay **TNF-gamma** is passed over a solid support and binds to antibody attached to a solid support. A second antibody is then bound to the **TNF-gamma**. A third antibody which is labeled and specific to the second antibody is then passed over the solid support and binds to. . . .

Example 1
Bacterial Expression and Purification of **TNF-Gamma**
The DNA sequence encoding **TNF-gamma**, ATCC # 75927, is initially amplified using PCR oligonucleotide primers corresponding to the 5' sequences of the **TNF-gamma** protein and the vector sequences 30 to the **TNF-gamma** gene.

Additional nucleotides corresponding to **TNF-gamma** were added

to the 5' and 31 sequences respectively. The 5' oligonucleotide primer has the sequence 50 GCGCGGATCCACCATGAGACGL7.riviTTAAGCAAAGTC 31 contains a Bam HI restriction enzyme site followed by the first 24 nucleotides of **TNF-gamma** coding sequence starting from the presumed terminal amino acid of the processed protein codon. The 3' sequence 51' CGCGTCTAGACTATAGTAAGAAGGCTCCAAAGAAGG 31 contains complementary sequences to an XbaI site and is followed by 22 nucleotides of **TNF-gamma** and to a pQE-9 vector sequence located 31 to the **TNF-gamma** DNA insert. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen). pQE-9 was then digested. . . Cells were then harvested by centrifugation. The cell pellet was solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized **TNF-gamma** was purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). **TNF-gamma** was further purified by a second run on the Nickel-chelate column. **TNF-gamma** (90% pure) was eluted from the column in molar guanidine HCl pH 5.0 and for the purpose of renaturation was dialyzed in. . . where M is molecular weight markers; lane 1 is induced cell lysate; lane 2 is uninduced cell lysate; lane 3 is the **TNF-gamma** protein after two Nickel-chelate column purifications; lane 4 is the **TNF-gamma** protein after 1 column purification.

Example 2

Cloning and expression of **TNF-gamma** using the baculovirus expression system

The DNA sequence encoding the full length **TNF-gamma** protein, ATCC 75927, was amplified using PCR oligonucleotide primers corresponding to the 51 and 31 sequences of the gene.

primer has the sequence 50 GCGCGGATCCACCATGAGACGCTTTTAAAGCAAAGTC 31 and contains a Bam HI restriction enzyme site (in bold) followed by 24 nucleotides of the **TNF-gamma** gene (the initiation codon for translation 11ATG11 is underlined).

50 CGCGTCTAGACTATAGTAAGAAGGCTCCAAAGAAGG 3' and contains the cleavage site for the restriction endonuclease XbaI and 22 nucleotides complementary to the 31 non-translated sequence of the **TNF-gamma** gene. The amplified sequences were isolated

from a 1% agarose gel using a commercially available kit (Geneclean, 11 BIO 101 Inc. ,. . .

The vector pA2 (modification of pVL941 vector, discussed below) is used for the expression of the **TNF-gamma** protein using the baculovirus expression system (for review see.

5 Ag of the plasmid pBac **TNF-gamma** was cotransfected with 1.0 Mg of a commercially available linearized baculovirus (BaculoGold baculovirus DNA, Pharmingen, San Diego, CA.) using the lipofection method (Felgner. . .

1Ag of BaculoGold' virus DNA and 5 jig of the plasmid pBac **TNF-gamma** were mixed in a sterile well of a microtiter plate containing 50 Al of serum free Grace's medium (Life Technologies Inc -. . .

Sf 9 cells were grown in Grace Is medium supplemented with 10% heat-inactivated FBS. The cells were infected with the recombinant baculovirus V-**TNF-gamma** at a multiplicity of infection (MOI) of 2. Six hours later the medium was removed and replaced with SF900 II medium minus. . . the labelled proteins visualized by SDS-PAGE and autoradiography. Figure 5 illustrates the gel where Lanes 1 and 3 are the medium of the **TNF-gamma** and control; lanes 2 and 4 are the cell lysate of the **TNF-gamma** and the control.

Exa=1e 3

E2Mression of Recombinant TNF-cFamma in COS cells
The expression of plasmid, **TNF-gamma** HA is derived from

a vector pcDNAI/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CKV promoter followed by a polylinker region, a SV40 intron and polyadenylation site. A DNA fragment encoding the entire **TNF-gamma** precursor and a HA tag fused in frame to its 3f end was cloned into the polylinker region of the vector, therefore,. . .

The DNA sequence encoding **TNF-gamma**, ATCC # 75927, was constructed by PCR on the original EST cloned using two primers: the 5F primer (same as for the bacula example) contains a BamHI site followed by 24 nucleotides of **TNF-gamma** coding sequence starting from the initiation codon; the 31 sequence 51 CGCTCTAGATCAAGCGTAGTCTGGGACGTCGTATGGATAGTAAGAAG GCTCCAAAG 3' contains complementary sequences to XbaI site, translation stop codon, HA tag and the last 18 nucleotides of the **TNF-gamma** coding sequence (not including the stop codon) .

Therefore, the PCR product contains a BamHI site, **TNF-**

gamma

coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag, and an XbaI site.. . . DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant **TNF-gamma**, COS cells were transfected with the expression vector by DEAE-DEXTRAN method. (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the **TNF-gamma** HA protein was detected by radiolabelling and immunoprecipitation method.

Example 4

Expression pattern of **TNF-gamma** in human tissue
RNA blot analysis was carried out to examine the levels of expression of **TNF-gamma** in human tissues. Total cellular RNA samples were isolated with RNeasy RBB system (Qiagen Laboratories, Inc. 6023 South Loop East, Houston, TX. . .

Maniatis, Molecular Cloning, Cold Spring Harbor Press, (1989)). The labeling reaction was done according to the Stratagene Prime-It kit with 50 ng **TNF-gamma** cDNA, to produce 32p -labeled **TNF-gamma** cDNA. The labeled DNA was purified with a Select-G-50 column (5 Prime - 3 Prime, Inc. 5603 Arapahoe Road, Boulder, CO 80303). The filter was then hybridized with radioactive labeled full-length **TNF-gamma** gene at 1,000,000 cpm/ml in 0.5 M NaPO₄. pH 7.4 and 7% SDS overnight at 65°C. After being washed twice at. . . with 0.5 x SSC, 0.1% SDS, the filter was then exposed at -70°C overnight with an intensifying screen. The message RNA for **TNF-gamma** is abundant in kidney (Figures 3A).

3B, with the only change being that 10 Mg poly A RNA from the tissues indicated were used. The message RNA for **TNF-gamma** is expressed predominantly in HUVEC cells (Figure 3B).

Example 5

Ability of Recombinant **TNF-gamma** to inhibit WEHI 164 and L929 cell growth, induce cell adhesion in HL-60 cells and promote endothelial cell growth.

plates containing 0.1 ml serially diluted test samples of cells (WEHI 164 and L929). Incubation was continued for 70 hours. **TNF-a**, **TNF-f1** and **TNF-gamma** were added at a 0.5 Ag/ml concentration. The cytotoxicity and proliferation activity was quantified using

an MTS assay performed by the addition. . . % cytotoxicity = $(100 - \text{OCexpen} = \text{nul}/\text{ODc},, \text{u}, 1) \times 100$. The photographs were taken after 72 hours. As shown by Figure 6A, and 8 **TNF-gamma** induced a morphology change which appeared as dark round cells which are killed.

graph of Figure 6B. the assay was performed as described above, however, increasing amounts of TNF were added. The results indicate that **TNF-gamma** is an inhibitor of WEHI 164 cells.

To test adhesion ability of **TNF-gamma**, HL-60 cells were used and cell adhesion and cell-cell contact were measured by observation under the microscope and scored subjectively by two independent investigators. Figure 10 illustrates **TNF-gamma**'s ability for inducing cell adhesion.

In the assay to test for ability of **TNF-gamma** to promote endothelial cell growth, the proliferation index (PI) was calculated as follows: $\text{PI} = \text{Mxmrml}/\text{Mcomrol}$. Figure 8 illustrates that **TNF-gamma** is a promotor of endothelial cell growth.

(eg., blocking solution). During the second incubation step, the nucleosomes contained in the WEHI 164 cell sample treated with the TNF-a, TNF-O or **TNF-gamma** bind via their histone components to the immobilized anti-histone antibody. In the third incubation step, anti-DNA-peroxidase (POD) reacts with the DNA-part of the. . . are indicated as the absorbance A405nm/A490. (See Boehringer mannheim Catalogue, 0990 C 93 2 1541170) (see Figure 7)

Exanwle 7

Receptor binding assay using **TNF-gamma**
TNF-a and **TNF-gamma** were Ni-NTA affinity chromatography purified using the 6-His tag and 1 Ag/well was added to a nickel chelate coated 96-well plate (Xenopore. . .

OD was measured using an ELISA reader (test wavelength 450 nm, correction wavelength 590 nm). The results shown in Figure 11 illustrate that **TNF-gamma** does not bind significantly to sTNF-receptors.

(ii) TITLE OF INVENTION: Human **Tumor Necrosis Factor-Gamma**

(iii) NUMBER OF SEQUENCES: 2

UV) CORRESPONDENCE ADDRESS.

CLMEN. . . cells with the

vector of Claim 9.

. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having **TNF-gamma** activity.

19 A method for the treatment of a patient having need of **TNF-gamma** comprising: administering to the patient a therapeutically effective amount of the polypeptide of claim 14.

20 A method for the treatment of a patient having need of **TNF-gamma** comprising: administering to the patient a therapeutically effective amount of the agonist of claim 17.

21 A method for the treatment of a patient having need to inhibit **TNF-gamma** comprising: administering to the patient a therapeutically effective amount of the antagonist of Claim 18.

.
polypeptide of claim 14 comprising:
combining endothelial cells, Concavilin-A, the compound to be screened, [3H] thymidine selectively in the presence or absence of **TNF-gamma**;
measuring the [3 H]thymidine incorporation by the endothelial cells; and
determining if the compound enhanced or blocked [3 H]thymidine incorporation.

. A method for inhibiting tumor cell growth in a patient comprising:
administering to the patient a therapeutically effective amount of the **TNF-gamma** protein optionally in the presence of a pharmaceutically acceptable carrier.

.
A method for diagnosing a tumor or a susceptibility to a tumor comprising:
detecting a mutated form of the nucleic acid sequence encoding **TNF-gamma** in a sample derived from a host.

=>

L7 ANSWER 9 OF 13 MEDLINE

ACCESSION NUMBER: 2001175167 MEDLINE

DOCUMENT NUMBER: 21170294 PubMed ID: 10973284

TITLE: **APRIL** and TALL-I and receptors BCMA and TACI:
system for regulating humoral immunity.

AUTHOR: Yu G; Boone T; Delaney J; Hawkins N; Kelley M;
Ramakrishnan

CORPORATE SOURCE: M; McCabe S; Qiu W R; Kornuc M; Xia X Z; Guo J; Stolina M;
Boyle W J; Sarosi I; Hsu H; Senaldi G; Theill L E
Department of Inflammation, Amgen Inc., One Amgen Center
Drive, Thousand Oaks, CA 91320-1799, USA.

SOURCE: Nat Immunol, (2000 Sep) 1 (3) 252-6.
Journal code: DOG; 100941354. ISSN: 1529-2908.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200104

ENTRY DATE: Entered STN: 20010410
Last Updated on STN: 20010410
Entered PubMed: 20010328
Entered Medline: 20010405

AB We report that the tumor neurosis factor homolog APRIL (a
proliferation-inducing ligand) stimulates in vitro proliferation of
primary B and T cells and increases spleen weight due to accumulation of

B cells in vivo. APRIL functions via binding to BCMA (B cell maturation
antigen) and TACI (transmembrane activator and CAML-interactor) and
competes with TALL-I (also called BLyS or BAFF) for receptor binding.
Soluble BCMA and TACI specifically prevent binding of APRIL and block
APRIL-stimulated proliferation of primary B cells. BCMA-Fc also inhibits
production of antibodies against keyhole limpet hemocyanin and Pneumovax
in mice, indicating that APRIL and/or TALL-I signaling via BCMA and/or
TACI are required for generation of humoral immunity. Thus, APRIL-TALL-I
and BCMA-TACI form a two ligands-two receptors pathway involved in
stimulation of B and T cell function.

L7 ANSWER 10 OF 13 MEDLINE

DUPLICATE 5

ACCESSION NUMBER: 1998416181 MEDLINE

DOCUMENT NUMBER: 98416181 PubMed ID: 9743536

TITLE: **APRIL**, a new ligand of the **tumor necrosis** factor family, stimulates tumor cell growth.

AUTHOR: Hahne M; Kataoka T; Schroter M; Hofmann K; Irmeler M; Bodmer

J L; Schneider P; Bornand T; Holler N; French L E; Sordat B; Rimoldi D; Tschopp J

CORPORATE SOURCE: Institute of Biochemistry, Lausanne Branch, University of Lausanne, Epalinges, Switzerland.

SOURCE: JOURNAL OF EXPERIMENTAL MEDICINE, (1998 Sep 21) 188 (6) 1185-90.

Journal code: I2V; 2985109R. ISSN: 0022-1007.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-AF046888

ENTRY MONTH: 199810

ENTRY DATE: Entered STN: 19981021

Last Updated on STN: 20000303

Entered Medline: 19981015

AB Members of the **tumor necrosis** factor (**TNF**)

family induce pleiotropic biological responses, including cell growth, differentiation, and even death. Here we describe a novel member of the **TNF** family designated APRIL (for a proliferation-inducing ligand).

Although transcripts of APRIL are of low abundance in normal tissues, high

levels of mRNA are detected in transformed cell lines, and in human cancers of colon, thyroid, and lymphoid tissues in vivo. The addition of recombinant APRIL to various tumor cells stimulates their proliferation. Moreover, APRIL-transfected NIH-3T3 cells show an increased rate of tumor growth in nude mice compared with the parental cell line. These findings suggest that APRIL may be implicated in the regulation of tumor cell growth.